Serum Xylosyltransferase Activity in Diabetic Patients as a Possible Marker of Reduced Proteoglycan Biosynthesis

CHRISTIAN GÖTTING, PHD
JOACHIM KUHN, PHD
KNUT KLEESIEK, MD

OBJECTIVE — Proteoglycan metabolism is altered in diabetic patients. The xylosyltransferases (XTs) are the initial and rate-limiting enzymes in the biosynthesis of the glycosaminoglycan chains in proteoglycans. Here, we analyzed whether the changed proteoglycan metabolism leads to altered serum XT levels in diabetic patients.

RESEARCH DESIGN AND METHODS — Serum XT activity was determined in 100 diabetic patients and 100 blood donors using a novel high-performance liquid chromatography electrospray ionization tandem mass spectrometry assay.

RESULTS — Serum XT activities in male and female diabetic patients were significantly decreased compared with those in the corresponding normoglycemic control subjects (mean ± SD: male patients, 19.3 ± 4.44 mU/l; male nondiabetic control subjects, 26.6 ± 2.79 mU/l; female patients, 18.9 ± 3.14 mU/l; female nondiabetic control subjects, 21.8 ± 3.74 mU/l; $P < 0.0001$). No significant differences were detected between patients with type 1 and type 2 diabetes.

CONCLUSIONS — Our data show decreased XT activity in patients with diabetes, a disease that is accompanied by an altered proteoglycan biosynthesis.

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Diabetic patients have been previously reported as having an altered proteoglycan metabolism, which results in decreased content of heparan sulfate proteoglycans in basement membranes (1). Proteoglycans consist of a core protein to which glycosaminoglycan chains are attached. The biosynthesis of the glycosaminoglycan chains is initiated by a xylosylation of the proteoglycan core protein. Xylosyltransferase (XT)-I and -II (enzyme commission [EC] no. 2.4.2.26) catalyze the initial and rate-limiting step in glycosaminoglycan biosynthesis and have been shown to play an important role in proteoglycan homeostasis (rev. in 2). XT-I and XT-II are Golgi-resident proteins that are shed from the Golgi membrane and are secreted into the extracellular space attached to large proteoglycans. Consequently, XT activity present in blood has been proposed as a biochemical marker for the determination of an enhanced proteoglycan biosynthesis rate (2).

Hyperglycemia has been shown to affect a series of cellular processes including glycosylation (3,4). In diabetic patients, the biosynthesis of heparan sulfate proteoglycans has been found to be decreased (5,6), and a reduced heparan sulfate glycosaminoglycan content has been reported in the arteries of diabetic patients (7). Furthermore, the association between diabetes and heparan sulfate glycosaminoglycans and impaired organ function has been intensively studied in the kidneys, where heparan sulfate proteoglycans are important components of the glomerular basement membrane (8,9).

From the Institut für Laboratoriums-und Transfusionsmedizin, Herz-und Diabeteszentrum Nordrhein-Westfalen, Universitätsklinik der Ruhr-Universität Bochum, Bad Oeynhausen, Germany.
Corresponding author: Christian Götting, cgoetting@hdz-nrw.de.
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RESEARCH DESIGN AND METHODS

— The study cohort comprised 100 unrelated Caucasian diabetic patients (50 male, aged 53 ± 8 years; 50 female, aged 54 ± 7 years) and 100 unrelated age- and sex-matched blood donors with normal blood glucose levels (3.9–5.6 mmol/l). Within the patient cohort, 35 were type 1 diabetic (17 male, aged 26 ± 12 years; 18 female, aged 29 ± 11 years) and 65 patients were type 2 diabetic (33 male, aged 56 ± 9 years; 32 female, aged 57 ± 12 years). The definition of type 1 and type 2 diabetes was according to current American Diabetes Association and World Health Organization recommendations (10). Disease duration was at least 5 years in all patients. The experimental design was approved by the local ethics committee, and all patients gave informed consent. Blood was drawn after overnight fast, and determination of XT activity was performed as described previously (11). Statistical analysis was performed using the Student's *t* test and the Kolmogoroff-Smirnoff test where appropriate. Normality testing for Gaussian distribution of values was performed using the *F* test, and multiple linear regression analyses were used to assess the independent role of the serum XT activity as well as sex, age, A1C, duration of diabetes, and other serum parameters (alanine aminotransferase, aspartate aminotransferase, bilirubin, calcium, cholesterol, cholinesterase, C-reactive protein, creatine kinase, creatinine, γ-glutamyl transferase, HDL cholesterol, lactate dehydrogenase, LDL cholesterol, potassium, sodium, total protein, triglycerides, urea, and uric acid). *P* values <0.05 were considered statistically significant.

RESULTS

— XT activities in male diabetic patients ($n = 50$) were significantly reduced in comparison with those in the control group ($P < 0.0001$). The mean ± SD (90% range) was 19.3 ± 4.44 mU/l (13.2–26.6) in male diabetic patients and 26.6 ± 2.79 mU/l (18.1–29.1) in nondiabetic male control subjects ($n = 50$, respectively) (Fig. 1). XT activities in serum specimens from female diabetic patients were also significantly decreased compared with those from nondiabetic female control subjects ($P < 0.0001$). In female

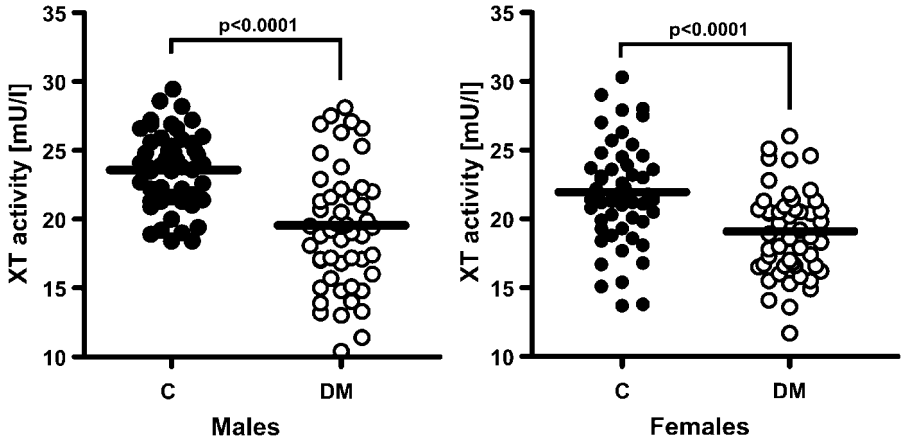

Figure 1—*Serum XT activities in male and female patients with diabetes (DM) and age- and sex-matched control subjects (C). The black bar represents the mean value. The XT activities of the male and female diabetic patients were significantly reduced compared with those of the corresponding control cohort ($P < 0.0001$).*

diabetic patients ($n = 50$), the mean ± SD (90% range) was 18.9 ± 3.14 mU/l (14.6–24.4) (Fig. 1). In the corresponding group of female nondiabetic control subjects ($n = 50$), the mean ± SD XT activities (90% range) was calculated as 21.8 ± 3.74 mU/l (15.3–29.2). The serum XT activities did not differ between patients with type 1 (19.9 ± 3.60 mU/l) and type 2 diabetes (18.7 ± 3.91 mU/l). The observed differences in serum XT activities remained significant after adjustment for sex, age, A1C, duration of diabetes, and other serum parameters as listed above. Furthermore, no significant correlation of the serum XT activities and these parameters was observed.

CONCLUSIONS — In the diabetic state, a reduced biosynthesis of proteoglycans has been described (5,6,12). A decrease in the glycosaminoglycan content has been reported for multiple tissues including arteries (7), glomerular basement membranes, or the endothelium (12). The associations between hyperglycemia, glycosaminoglycan concentration, and impaired organ function have been well studied in the kidneys (1). These alterations point to those enzymes involved in the biosynthesis of the glycosaminoglycan chains and to both their regulation and enzymatic activity as potential modifiers of this process.

XT-I and XT-II catalyze the initial and rate-limiting transfer of xylose to selected serine residues of the proteoglycan core protein (2). Both enzymes are shed from the Golgi membrane and are released into the extracellular space attached to large proteoglycans (2,13). Therefore, the XT activity in body fluids reflects the actual proteoglycan biosynthesis rate. While the biological role of XT secretion is not understood, the quantification of XT activity in the peripheral blood and other body fluids could be successfully validated as a marker of the actual proteoglycan biosynthesis rate (2).

In the present study, we show for the first time reduced XT activity in diabetes, a disease in which a reduced proteoglycan biosynthesis rate has been demonstrated (7,8,12). This low XT activity is proposed to result from a decreased enzyme biosynthesis rate or a lowered release from the Golgi apparatus; however, an increase of enzyme turnover or an elimination of the enzyme from the blood stream must also be taken into account. Furthermore, there is a significant overlap in the XT activities of diabetic patients and normal subjects pointing to multiple factors affecting the individual serum XT activity. The XT activity determined in peripheral blood is supposed to be a mixture of XT-I and XT-II enzyme activity, as both enzymes are released into the extracellular space and share highly similar acceptor specificity. To date, neither immunologic nor enzyme activity assays that are suitable for discriminating between the two XT isoforms are available. The future development of XT-I – and XT-II–specific assays will help to elucidate whether diagnostic advancement is achieved by a selected determination of the XT isoforms.

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References

1. Vernier RL, Steffes MW, Sisson-Ross S, Mauer SM: Heparan sulfate proteoglycan in the glomerular basement membrane in type 1 diabetes mellitus. *Kidney Int* 41: 1070–1080, 1992
2. Götting C, Kuhn J, Kleesiek K: Human xylosyltransferases in health and disease. *Cell Mol Life Sci* 64:1498–1517, 2007
3. Yamagishi S, Imaizumi T: Diabetic vascular complications: pathophysiology, biochemical basis and potential therapeutic strategy. *Curr Pharm Des* 11:2279–2299, 2005
4. Brownlee M: Biochemistry and molecular cell biology of diabetic complications. *Nature* 414:813–820, 2001
5. Jensen T: Pathogenesis of diabetic vascular disease: evidence for the role of reduced heparan sulfate proteoglycan. *Diabetes* 46 (Suppl. 2):S98–S100, 1997
6. Kjellen L, Bielefeld D, Hook M: Reduced sulfation of liver heparan sulfate in experimentally diabetic rats. *Diabetes* 32:337–342, 1983
7. Vogl-Willis CA, Edwards IJ: High-glucose-induced structural changes in the heparan sulfate proteoglycan, perlecan, of cultured human aortic endothelial cells. *Biochim Biophys Acta* 1672:36–45, 2004
8. Vogl-Willis CA, Edwards IJ: High glucose-induced alterations in subendothelial matrix perlecan leads to increased monocyte binding. *Arterioscler Thromb Vasc Biol* 24:858–863, 2004
9. Deckert T, Feldt-Rasmussen B, Borch-Johnsen K, Jensen T, Kofoed-Enevoldsen A: Albuminuria reflects widespread vascular damage: The Steno hypothesis. *Diabetologia* 32:219–226, 1989
10. Alberti KG, Zimmet PZ: Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1. Diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. *Diabet Med* 15:539–553, 1998
11. Kuhn J, Prante C, Schön S, Götting C, Kleesiek K: Measurement of fibrosis marker xylosyltransferase I activity by HPLC electrospray ionization tandem mass spectrometry. *Clin Chem* 52:2243–2249, 2006
12. Gharagozlian S, Borrebaek J, Henriksen T, Omsland TK, Shegarfi H, Kolset SO: Effect of hyperglycemic condition on proteoglycan secretion in cultured human endothelial cells. *Eur J Nutr* 45:369–375, 2006
13. Schön S, Prante C, Bahr C, Kuhn J, Kleesiek K, Götting C: Cloning and recombinant expression of active full-length xylosyltransferase I (XT-I) and characterization of subcellular localization of XT-I and XT-II. *J Biol Chem* 281: 14224–14231, 2006